2023 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 13, Issue 3: 192-198; May 27, 2023 ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2023.29

INFLUENCE OF DIETARY SUPPLEMENTATION OF ANTIBIOTIC AND THYME ON ZOOTECHNICAL PARAMETERS AND CAECAL MICROFLORA OF GROWING RABBIT

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Supporting Information

RESEARCH ARTICLE
PII: S222877012200029-13
Received: December 06, 2022
Revised: May 24, 2023
Accepted: May 25, 2023

ABSTRACT: The objective of this study was to compare the influence of antibiotic and thyme dietary supplements on zootechnical parameters and caecal microflora of growing rabbits. One hundred and ninety eight weaned rabbits (forty days old), white New Zealand (of both sexes) were divided into three groups to submit to the following dietary treatments: Control diet, diet A (control diet + 100 ppm zinc bacitracin), and diet T (control diet + 7% *Thymus capitatus* leaves) for twenty-one days. The remaining nine days they received only the control diet. The results showed that both the live body weight and feed conversion ratio were positively affected by the antibiotic diet (P<0.05). However, the rabbits' growth performance was not influenced by dietary thyme supplements. The antimicrobial effect of thyme observed against *C. perfringens* in caecum is not determined even after 20 days of treatment. In conclusion, zootechnical parameters and mortality were not positively affected by dietary thyme supplements comparing it with the antibiotic diet, but these phytobiotics showed the antibacterial effect against *E. coli* and *C. perfringens* in caecum of rabbit.

Keywords: Zinc bacitracin, Dietary supplementation, Growth performance, Rabbit, Thyme.

INTRODUCTION

The weaning period in the rabbit life can be very critical (20-40 days of age) due to the transition from milk to solid nutrition (Gidenne et al., 2010). Also, the development of a caecal microbial activity starts to stabilize only after 7 weeks of age (DI Meo et al., 2004). Moreover non-specific or specific enteropathy is always a major problem leading to great losses of rabbits (Abdeen et al., 2011; Conficoni et al., 2020). Furthermore, Phytobiotics are discussed as one of the promising alternatives to replace antibiotic growth promoters due to their high content of pharmacologically active compounds (Grashorn, 2010; Skoufos et al., 2020). The phytobiotics can prevent digestive disorders and optimize productivity (Krieg et al. 2009; Celia et al. 2016). They also improve health performance by increasing antioxidant activity in tissues (Settle et al. 2014; Abdel-Azeem and El-Kader, 2022) and enhancing immunity (Lee et al. 2010).

Among these phytobiotics, there are species of the Thymus genus, aromatic plants of the Lamiaceae family (Casiglia et al., 2019). Some studies have demonstrated the interesting biological properties of the Thymus (Soković et al., 2008; El-Nekeety et al., 2011; Nikolić et al., 2014). It has an antiseptic, antimicrobial, and antioxidant effect (Kaki et al., 2021; Pandey et al., 2021). Thyme improves liver function and acts as an appetite stimulant (Dauqan et al. 2017; Almanea et al., 2019).

The objective of the present study is to examine and compare the effects of antibiotic with *Thymus capitatus* as natural feed supplements on growth performance and caecal microflora in weaned rabbits.

MATERIAL AND METHODS

Ethical approval

According to Directive 2010/63/EU of 22 September 2010, and recommendation of the European Commission 2007/526/CE, the animal in the current study were used for experimental and other scientific purposes.

Medicinal plant supplementation

The areal parts of *Thymus capitatus* were collected in northern Morocco during the month of June. Identification was performed in the Laboratory by Professor Bakkali, a specialist in botany. Afterward, the leaves were separated and dried at room temperature for 2 weeks in the absence of light and then stored in sealed paper bags until their use.

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Animals and experimental procedure

A total of one hundred and ninety eight weaned rabbits (40 days old, white New Zeland of both sexes 900 ± 100 g initial weight), were divided into three groups and submitted to the following dietary treatments:

The control diet (in Table 1 formulation and chemical composition); the antibiotic diet (control diet + 100 ppm of Zinc bacitracin) and T diet (control diet + 7% *Thymus capitatus* leaves). Sixty-six rabbits per group. Rabbits were kept in in cages (L=90cm, W=40cm, H=35cm with six animals per cage) in a building with temperatures between 15 and 20°C. A total of 198 weaned rabbits were divided into three groups: 66 rabbit per group, and 66 rabbits there were in each replicate, per treatment). The length of daily illumination was 16 hours. The rabbits had access to feed and water ad libitum. The rabbits Body weight and feed intake were measured every week during the experiment as well as their mortality rates. Five animals from each group were randomly slaughtered at days 40, 50 and 60. Rabbits were weighed before and after slaughter and evisceration at days, 50, 60 and 70 to determine the yield of the carcass. The livers were weighed too.

Table 1 - Ingredients and chemical composition and nutritive value of diets in rabbits

Ingredients	Control diet (%)	T diet (%)	Chemical composition (g/100g)	Control diet	T diet
Wheat bran	28.5	26.505	Dry matter	89.9	89.7
Corn	9.5	8.835	Ash	7.7	7.8
Soybean meal	9.5	8.835	Crude protein	20.3	19.4
Sunflower meal	14.2	13.206	Ether extract	5.8	6.2
Alfalfa	33.75	31.3875	Neutral detergent fibre	30.5	30.6
Vegetable oil	2.8	2.604	Acid detergent fibre	17.5	18.25
Salt	0.5	0.465	detergent lignin	4.6	4.8
Premix *	0.6	0.558	Digestible energy (Kcal/Kg)	2522	2531
DL Methionine	0.1	0.093			
L-Lysine	0.2	0.186			
Dicalcium phosphate	0.25	0.2325			
Calcium carbonate	0.1	0.093			
Thyme leaves	0	7			

* One kilogram of Premix provides: 1000000 IU vit.A, 300000 IU vit. D, 2 g vit. E, 0.4 g vit. K, 0.075 g vit. B1, 0.4 g vit. B2, 1.218 g vit. B3, 0.099 g vit. B5, 0.083 g vit. B6, 0.190 g vit. B9, 0.030 g vit. B12, 0.005 g Biotin, 0,2 g Cuivre, 4 g Fer, 5 g Zinc, 0.012 g Iode, 0.012 g Selenium, 0.020g Cobalt, 6 g Manganese, 57 g Choline chloride and QSP calcium. Premix contained 50 ppm of Salinomycin.

Chemical analysis

Chemical analysis of diets were calculated by the Spanish foundation for the development of animal nutrition (Fundación Española para el Desarrollo de la Nutrición Animal, FEDNA) table of composition and nutritive valor of aliments (Blas et al. 2010).The essential oil of *Thymus capitatus* was analyzed with a gas chromatograph (Trace GC ULTRA; Thermo Scientific, Waltham, MA, USA) coupled to a mass spectrometer (Polaris Q MS with ion trap; Thermo Scientific) in the electron impact (EI) ionization mode (70 eV) in the 50–350 m/z range. The analysis was carried out using a VB-5 methylpolysiloxane at 5% phenyl) (Thermo Scientific) column (30m × 0.25mm, film-thickness 0.25 μ m) using a temperature program of 40–300◦ C at a 4◦C min−1. Injector temperature was set at 220◦C. Helium gas was used as the carrier gas at a constant flow rate of 1.4 ml min−1. Diluted samples (1% in n-hexane; Sigma–Aldrich, Steinheim, Germany) of 1.0 μL were injected in the split mode to allow better identification of compounds. The analysis was repeated twice for each sample. The constituents were identified by comparison of their retention indices and mass spectra with those in the computer library (NIST MS Library Search, v.6.0) and with literature data.

Bacteriological analysis

Bacteria from caecal samples were isolated by the standard microbiological method using the appropriate dilutions in Ringer solution. Dilutions were plated onto the following media: Mac Conkey agar for *E.coli*, incubated at 37°C for 24 h and agar Tryptose Sulphite added with antibiotic D-Cycloserine (TSC) for *C. perfringens* incubated during 48 hours at 37°C, with their enumeration determined according to the ISO 7937 standard (1997). The bacterial counts per colony were expressed in grams using this formula: (log10 CFU/g ± SD). Experiments were carried out in triplicate.

Statistical analysis

The results were quoted as mean ± standard deviation (SD), and statistical evaluation of the results was performed by the one-way ANOVA using the general linear model (GLM) procedures of SAS with the level of significance set at $p < 0.05$ and Square test for mortality.

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RESULTS

Live weight, growth rate, feed intake, feed conversion rate, carcass yield, and the mortality of rabbits during the experiment are presented in Table 2. Live body weight (BW) was positively affected by the antibiotic diet. The addition of 7% of thyme in the diet had a negative effect on the weight and growth rate of the young compared to other groups because they eat a smaller quantity of feed. Although the feed conversion rate did not differ significantly when the experimental period was considered as a whole. The rate of conversion for the antibiotic group tended to fare better during the first week of treatment.

There was a difference in the mean carcass yield of the thyme group on the first ten days of the treatment. A high mortality rate was observed in the three groups. Effects of an antibiotic and thyme dietary supplements on the caecal microbial colony-forming unit (CFU) are presented in Table 3. No significant effect was found on the caecal count of *E. coli* or *C. perfringens* due to antibiotic diet. The CFU of *E. coli* in digesta harvested from the caecum was also not influenced by dietary supplementation of medicinal plant. While it was observed the number of *C. perfringens* in the caecum of rabbits fed the thyme feed was low compared to the control and antibiotic group after 10 days of treatment and it could not even be determined after 20 days of treatment. The chemical composition of the essential oil of *Thymus capitatus* is presented in table 4. The yield of essential oil of *Thymus capitatus* was 1.96%. The *Thymus capitatus* oil was dominated by carvacrol (95.25%).

Table 2 - The Effect of dietary supplementation with thyme on rabbit growth performance and mortality.

Indices	Days	Group (mean ± SD)			
		Control diet	Antibiotic diet	Thyme diet	P value
Live weight (g)	40	910 ± 130	874 ± 125	893 ± 116	0.24 ^{NS}
	47	1183 ± 174 ^{a}	1279 ± 207 ^{b}	1171 ± 166 ^{a}	0.004**
	54	1391 ± 162 ^{a}	1514 ± 256 ^{b}	1336 ± 167 ^{a}	0.001**
	61	1623 ± 235 ^{a}	1765 ± 243 ^{b}	1550 ± 172 ^{a}	0.001**
	70	1972 ± 301 ^{ab}	2015 ± 253 ^{b}	1859 ± 202 ^{a}	0.008**
Feed intake (g/d)	40	86.8 ± 5 ^{b}	84.5 ± 6 ^{b}	78.5 ± 5 ^{a}	0.001**
	47	97 ± 12	96.5 ± 6	87.5 ± 7	0.754 ^{NS}
	54	109 ± 7	103,1 ± 13	99,2 ± 12	0.141 ^{NS}
	61	122.4 ± 13 ^{b}	110.2 ± 9a ^{b}	104.1 ± 12 ^{a}	0.002**
	70	137 ± 18	135.9 ± 18	119.8 ± 21	0.096 ^{NS}
Growth rate (g/d)	40-47	40 ± 16 ^{a}	51.7 ± 19 ^{b}	41.4 ± 13 ^{a}	0.001
	47-54	34.3 ± 12	34.8 ± 10	30.2 ± 9	0.144 ^{NS}
	54-61	36.6 ± 9	30.8 ± 9	30.3 ± 9	0.142 ^{NS}
	61-70	38.4 ± 11 ^{b}	26 ± 12 ^{a}	31.5 ± 10 ^{b}	0.002 **
Feed conversion ratio	40-47	2.56 ± 1	2.14 ± 1	2.37 ± 1	0.286 ^{NS}
	47-54	3.04 ± 0.7	2.98 ± 0.8	3.07 ± 0.7	0.915 ^{NS}
	54-61	3.45 ± 0.9	3.58 ± 0.9	3.47 ± 0.8	0.865 ^{NS}
	61-70	3.49 ± 0.7	3.9 ± 0.8	3.57 ± 0.8	0.323 ^{NS}
Carcass yield (% of BW)	50	52.1 ± 2 ^{b}	51.5 ± 1 ^{b}	48.3 ± 2 ^{a}	0.003**
	60	62 ± 7	63.4 ± 1	57.6 ± 1	0.189 ^{NS}
	70	53.4 ± 2	53.8 ± 1	51.8 ± 2	0.192 ^{NS}
Liver yield (% of BW)	50	3.1 ± 0,3	3.8 ± 0.4	3.4 ± 0.7	0.127 ^{NS}
	60	3.2 ± 0,4	3.1 ± 0.1	3.6 ± 0.4	0.182 ^{NS}
	70	4.1 ± 0.3	4 ± 0.2	4.2 ± 0.2	0.487 ^{NS}
Mortality[1] (%)	40-70	39.30%	40.90%	44.40%	> 0.05

[1] Mortality is analyzed by using a χ 2 test at P<0.05; The values with different superscript letters in a row are significantly different (p<0.05)

Table 3 - Counts of *E. coli* and *C. perfringens* in caecum of rabbits (log10 cfu/g (mean±SD)

Days	Bacteria	Group (mean ± SD)			
		Control diet	Antibiotic diet	Thyme diet	P value
40 d	*E. coli*	3.48 ± 0.2	-	-	-
	C. perfringens	2.89 ± 0.1	-	-	-
50 d	*E. coli*	4.1± 0.2	3.93 ± 0.5	4.2 ± 0.1	0.503 ^{NS}
	C. perfringens	2.92 ± 0.6	2.51 ± 0.4	2.24 ± 0.3	0.284 ^{NS}
60 d	*E.coli*	4.21 ± 0.7	4.2 ± 0.1	3.56 ± 0.8	0.157 ^{NS}
	C. perfringens	3.2 ± 0.3	2.71 ± 0.3	ND	0.209 ^{NS}

SD: Standard deviation; N.S: Non-significant at probability value (P>0.05). ND: Is not determined

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Table 4 - Chemical composition of *Thymus capitatus* essential oil.			
No.	Component	Retention time (min)	*Thymus capitatus*
1	Myrcene	6.55	-
2	Para cymene	7.19	-
3	cis-Ocimene	7.73	-
4	α-Pinene, (-)-	7.75	-
5	γ terpinene	7.79	-
6	α-Pinene, (-)-	8.28	-
7	Linalol	8.51	-
8	Camphene	8.74	-
9	α-Phellandrene	9.70	-
10	Terpinene -4-ol	9.79	-
11	α-Pinene, (-)-	11.01	-
12	dl-Limonene	11.01	-
13	Thymol	11.38	-
14	Carvacrol	11.55	-
15	1-8 cineol	11.68	-
16	Fenchone	12.99	-
17	Fenchone	13.00	-
18	β Caryophyllene	13.08	-
19	α Campholene Aldehyde	15.63	-
20	Borneol	16.47	-
21	Isopulegyl acetate	17.81	-
22	α-Fenchyl acetate	18.23	-
23	Trans Anethol	20.07	-
24	Trans Anethol	20.10	-
25	Carvacrol	20.69	95,25
26	Caryophyllene	24.24	1,49
27	Caryophyllene	24.89	-
28	Tetradecamethylcycloheptasiloxane	27.26	-
29	3,5-Diethylphenol	37.95	0.74
31	1,15-Dihydrohexadecamethyloctasiloxane	38.85	-
32	6-Acetyl-2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-1-benzopyran	39.37	0.91

* Analyzed by authors.

DISCUSSION

The weaning age affected growth performance and the caecal fermentation was strongly stimulated by the early weaning at 21 days of age (Xiccato et al., 2003). The crude protein of the rabbit diet recommended range was 14.5 – 16.2 g for fattening rabbits and 15.4 – 16.2 g in mixed feed Mateos and de Blas (1998). The crude protein of the rabbit diet was high in this experience, which can explain the high mortality rate of the three groups. Mortality, clinical symptoms, and performance data were significantly improved by zinc bacitracin soluble powder in the early treatment of Epizootic Rabbit Enteropathy in rabbits reared under normal field conditions (Maertens et al., 2005). According to Benlemlih et al. (2020) dietary supplementation with 5% dried thyme improved rabbit performance. The treatment with 2.5% of fennel and thyme had also a beneficial effect and decreased the significant mortality rate (Benlemlih et al., 2014). Abdel Wareth et al. (2018) proved that thyme essential oil included up to 100 mg/kg ration with 1.50 g/kg olive oil as carrier as an supplement in the ration of growing rabbits can be used as an effective feed additive to improve performance under hot environmental conditions. However, this experiment showed that supplementing the diet with 7% of thyme increase the mortality rate. Supplementing the diet with 3% thyme did not affect rabbit mortality (Gerencsér et al., 2012). Zinc bacitracin was tested and observed a significant improvement in weight gain (Boisot et al., 2004; Letlole et al., 2021; Martínez et al., 2022). However, in agreement with our observations, Pinheiro et al. (2004) found that the growth performances were not significantly increased ($P > 0.05$) by antibiotic supply during the growing period.

Carvacrol is the main ingredient of their essential oil, is known to evoke a sense of warmth, and sensitize skin by activating the transient receptor potential channel (TRPV3) (Xu et al. 2006). So the higher quantity of thyme leaves in dietary participated in reducing the amount ingested. That makes it clear why the decrease in rabbit consumption, affects weight and growth rate.

Rabbits fed Zinc bacitracin and FormaXol diets harbored the highest percentage of non-pathogen bacteria (Cardinali et al., 2008). The concentration of different bacterial populations on caecal content decreased significantly in growing

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rabbits with an antibiotic growth performance diet. Coliform population was reduced in half and the total bacteria count by 26% (Pinheiro et al. 2004).

In disagreement with our observation, rabbit feed supplemented with 3% of thyme did not affect the caecal number of *E.coli*, total anaerobic, and strictly anaerobic bacteria (Bónai et al. 2012). But essential oil extracted from thyme and anise have been reported to decrease *C. perfringens* and *E. coli* counts in both small and large intestines, accompanied by decreased intestinal lesion scores in broilers (Cho et al. 2014). The changes in intestinal microbiota might be related to the alleviation of intestinal lesions with essential oil supplementation (Du et al. 2015).

The antibacterial activity of the individual oil component was tested by the component with the widest spectrum of activity was found to be thymol followed by carvacrol (Dorman and Deans 2000). So, the high dose of carvacrol in our phytobiotic may have reduced the number of *C. perfringens* in the caecum which has not allowed us to determine its number. Aromatherapy rabbits with thyme essential oil can be suggested to treat diarrhea and bacterial enteritis.

Carvacrol can destabilize the cytoplasmic membrane and acts as a proton exchanger. Thereby reducing the pH gradient across the cytoplasmic membrane, the resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death (Ultee et al., 2002).

CONCLUSION

The addition of a high dose of phytobiotics (7% of *Thymus capitatus*) gave negative results on different zootechnical parameters and mortality comparing it with the antibiotic. But the active compounds of phytobiotic were responsible for antibacterial effects. The presence of carvacrol in thyme essential oil can intervene in the reduction of the number of *E. coli* and *C. perfringens* in the caecum. The addition of a moderate dose of *Thymus capitatus* should be stadied.

DECLARATIONS

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Acknowledgments

The corresponding author thanks National Center for Scientific and Technical Research for the research grant. The authors would like to thank the members of the rabbit company Lapinord for co-operation in this experiment and we gratefully thank the reviewers for their comments on this manuscript.

Authors' contribution

All authors contributed to research conduction, analyzing and writing, equally.

Conflict of interests

The authors declare that there is no conflict of interests in this work.

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